

AN EXPERIMENTAL STUDY OF SIMULTANEOUS VACCINATION AGAINST TULAREMIA AND  
TUBERCULOSIS

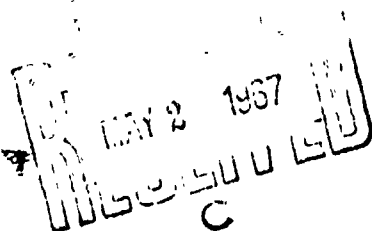
AD 650894  
TT 67-61628

Translation No. 1668

ARCHIVE COPY

May 1966

U. S. ARMY  
BIOLOGICAL LABORATORIES  
FORT DETRICK, FREDERICK, MARYLAND



## AN EXPERIMENTAL STUDY OF SIMULTANEOUS VACCINATION AGAINST TULAREMIA AND TUBERCULOSIS

[Following is the translation of an article by R. A. Savelyeva, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No 4, 1964, pages 118--124. It was submitted on 18 Jul 1962. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

At the present time there are many reports dealing with the study of simultaneous vaccination against tularemia and other infections (Pilipenko and Polyakova, 1955, 1956; Gubina, 1957; Shlygina, 1958; Gubina and Uglovoy, 1958; Amanzhulov and Rementsova, 1958; Borodko et al., 1959; Kalacheva, 1960; Belyakov and Bobrovskiy et al., 1960).

The Soviet Union is one of the few countries where inoculations against tuberculosis are compulsory. Yearly we inoculate around 10 million persons against tularemia. Therefore attempts at the simultaneous vaccination against these two infections are understandable. It is true that the first inoculation against tularemia, which is given for the first time when the person is 7 years old, would coincide with the second revaccination against tuberculosis.

The advantages of a simultaneous vaccination in this case are the same as during the combined vaccination against other infections. Besides this, both vaccines are mildly reactogenic, are applied cutaneously, and the revaccinations are performed after lengthy intervals.

The mission of the present paper includes the study of the simultaneous (both separate and associated) cutaneous vaccination against tularemia and tuberculosis.<sup>1</sup>

Since there are certain differences in the method of vaccination against tuberculosis and tularemia, we considered it necessary to set up the tests in different variations. In particular there are differences in the methods for the scarification of the skin during the application of this or that vaccine. During the administration of tularemia vaccine the scarifications are made on the skin with a vaccination quill, and during the administration of tuberculosis vaccine a superficial scarification is made with a special 4-serrate scarificator (1 x 3 cm in area).

Our mission included observations on the formation of immunity and the

<sup>1</sup>The inoculations against tuberculosis, and also the study of the immunity in animals to this infection, were in collaboration with fellow scientist M. L. Khatenever.

intensity of it in animals against tularemia.<sup>2</sup>

The tests were carried out on healthy guinea pigs weighing 300--400 grams. The animals (2 groups of 15 animals each) were vaccinated simultaneously, but separately, against tularemia and tuberculosis. For the vaccination of animals in the first group we used the dry BCG vaccine (100 mg in 1 ml) and tularemia vaccine series No 720, prepared at the Gamaleya Institute of Epidemiology and Microbiology; the animals of the second group were vaccinated with liquid BCG vaccine (30 mg in 1 ml) and one billion tularemia microbes of strain No 15 (attenuated). Each group had the corresponding controls, which were made up of the same number of pigs which had been vaccinated with monovaccines. The inoculations with the above named vaccines were made on the same shaved side of the guinea pig. This involved the lymph nodes of one side in the vaccinal process. Subsequently, for the purpose of checking the intensity of immunity we infected the pigs in the opposite inguinal area. We carried out the vaccination against tularemia by means of making two parallel incisions through a drop of vaccine in two places. The incisions were made up until the appearance of very minute droplets of blood (not at once), with the subsequent rubbing in of the vaccine for approximately a minute. The special 4-serrate scarificator was used for vaccination against tuberculosis.

After the vaccination the temperatures of the animals were systematically taken, they were weighed periodically and the local inoculation reaction was carefully recorded. On the 21-22nd day following vaccination the agglutination reaction was set up with blood sera from the animals and a tularemia diagnostic agent<sup>3</sup>, since it is known that the agglutination titer reaches the maximum by this time. An analogous reaction was set up prior to the control infection of the animals, that is, on the 42--45th day. During these same periods we set up an intracutaneous allergic probe with various dilutions of tularin.

As a result of the vaccination against tularemia a distinct local inoculation reaction was observed in all cases -- hyperemia, infiltration (1cm in diameter), scabs which subsequently fell off on the 8th day. Following the administration of the BCG vaccine we observed a very light hyperemia and infiltration of the skin in the first 2--3 days along with the dropping off of the very soft superficial scabs. The reaction of the regional lymph nodes was moderate and did not take place in all cases. In all the pigs there was an increase in temperature up to 41° for a period of 3-5-7 days. There were no significant changes in weight. After 3 weeks in all the pigs of the first group (vaccinated with dry BCG vaccine and tularemia vaccine series No 720) the agglutination reaction with the tularemia diagnostic agent was positive in dilutions of 1:10--1:640 (average of 1:244). In the

<sup>2</sup>For the results of the study of immunity to tuberculosis, see the article by M. L. Khatenever (Zh. mikrobiol., 1964, No 2).

<sup>3</sup>The diagnostic agent was prepared from the attenuated culture No 7, which belongs to the American variety of the tularemia bacterium.

control group, that is, in animals vaccinated only against tularemia, the titer fluctuated from 1:40 up to 1:320 (an average of 1:187). After 3 weeks in the second group, that is, in animals vaccinated with the liquid BCG vaccine and strain No 15 (attenuated), the agglutination reaction was positive in all the animals in dilutions of 1:80--1:640 (an average of 1:400). In the control group the titers fluctuated from 1:80 up to 1:640 (an average of 1:320). In both test groups the titer of the sera was noticeably higher than in the control, and the highest serum titer proved to be in animals which were vaccinated simultaneously with the liquid BCG vaccine and strain No 15 (reduced). A lowering of the serum titer took place after 42 days. This is quite usual (table 1).

For setting up the allergic probe we used fractional doses of tularin in order to determine the threshold of cutaneous sensitivity. We administered 0.1 ml of tularin to the pigs in the following concentrations: 1 billion, 100 million and 1 million microbial cells in 1 ml. The allergic reaction was positive in all the pigs without exception (both the test and the control) to all the dilutions of tularin. The intensity and dimensions of the cutaneous reaction fully reflected the concentration of tularin with its maximum manifestation after 24 hours. The allergic reaction in the test animals was more expressed than in the control. Thus, following the administration of a 1-billion suspension of tularin, the cutaneous reaction in the test animals reached 2--2.2 cm in diameter, and in the control 1.3 -- 1.4 cm. This regularity was also preserved with the least concentrations of tularin,. Besides this, for the greatest number of test animals necrosis was observed in the center.

After 2-3 days following the setting up of the allergic probe, all the animals (12 in the first group, 12 in the second, and 12 and 15 correspondingly in the controls to them) were infected subcutaneously with 1000 Dclm of a virulent strain of the tularemia microbe (No 503). During the subcutaneous administration of this strain to white mice and guinea pigs, 1 Dclm comprised 1 microbial cell based on the optical standard. As a result of infection one pig in each test group died on the 19th and on the 23rd day. Upon autopsy of the dead animals the pathologoanatomical picture which is characteristic for tularemia infection was detected and the causative agent was isolated. All the control pigs, which were vaccinated only against tularemia, survived, and 5 nonvaccinated healthy pigs, which were infected with an analogous dose of the virulent strain (control for the infection), died from tularemia on the 6--8th day. Not one of the surviving pigs lost weight following infection and only 3--4 pigs in each group had fever within a week. In this manner, the tests showed that following the simultaneous vaccination against tularemia and tuberculosis an immunity of high intensity against tularemia infection was developed.

The skin from the sites of application of the vaccine, the regional and nonregional lymph nodes, and also the spleen of the test pigs were subjected to a histological investigation. The changes revealed bore a benign nature and in essence were no different from changes observed in pigs which

were inoculated with monovaccines<sup>4</sup>.

Having in mind the necessity for the subsequent study of the effectiveness of associated vaccination against these two infections, we wanted to investigate the peculiarities in the development of immunity against tularemia by inoculating the animals with the special 4-serrate scarificator which is used during vaccination against tuberculosis. We used this scarificator and various concentrations of a vaccine strain of the tularensis organism -- 1 billion, 100 million and 10 million in 1 ml. After 1--2 days the local reaction in the pigs was distinct for the inoculation of all the concentrations of vaccine. A particularly clear reaction was observed following the administration of a suspension with a count of 1 billion; the dimensions of hyperemia and infiltration of the skin went beyond the limits of the scarified area. The agglutination reaction after 21 days, and after 42 days, was in the highest titer also in the animals which were vaccinated with a suspension having a density of 1 billion (table 2). Following infection with 1000 Dclm the death of pigs was observed in the group which was inoculated with 10 million microbial cells of a vaccine strain. Of the surviving pigs, all those vaccinated with 100 and 10 million microbial cells had fever (during the course of 4--14 days up to 41°) as a result of infection, and of those vaccinated with 1 billion cells only 3 had fever; none of the pigs lost weight.

Consequently, with the utilization of the special 4-serrate scarificator, the inoculation of animals against tularemia turned out successfully and led to the formation of a high immunity only following the administration of a suspension of a vaccine strain with a density of 100 million and 1 billion.

Subsequently we studied the effectiveness of associated vaccination against tularemia and tuberculosis. For this we used the same vaccines and doses as in the first part of the investigations. We used the same methods for the vaccination of the animals -- in 10 animals the skin was scarified with the 4-serrate scarificator, and in 8 - with a vaccination quill. Each group had the corresponding control animals which were inoculated with monovaccines. Prior to application on the skin the monovaccines which were prepared for associated immunization were mixed in equal volumes.

After 24 hours, in all the test and control pigs which had been inoculated with the 4-serrate scarificator, hyperemia and infiltration were noted at the site of administration of the vaccine. These dimensions subsequently exceeded the dimensions of the scarified sector of the skin. The local inoculation reaction lasted for approximately a week and then gradually faded away. The regional lymph nodes reacted to the inoculation moderately and only in half of the cases. Following the vaccination all the pigs had fever (40.7--41°) in the course of 3--5 days (individual animals had fever

<sup>4</sup>The investigations were carried out with fellow worker from the laboratory for tuberculosis prophylaxis A. Ye. Chigirinskiy; the results will be described in detail by him in a separate communication.

for a longer period, apparently connected with the presence of pneumococcal infection). After 21 days following vaccination the titer of the sera (table 3) in the animals which were inoculated with the liquid BCG vaccine and strain No 15 turned out to be the highest - 1:40--1:640 (an average of 1:245); in the control group it fluctuated from 1:40 to 1:320 (an average of 1:163). After 21 days the group inoculated with the dry BCG vaccine and one of the series of tularemia vaccine (No 802) had agglutination titers equaling 1:40--1:320 (an average of 1:143), and in the control group - 1:40 -- 1:320 (an average of 1:187). In the event of associated vaccination by using the method of incisions which is accepted with tularemia, after 21 days the agglutination reaction was also positive in dilutions from 1:40 to 1:320 (an average of 1:126) following the use of the liquid BCG vaccine and strain No 15, and in the event of inoculation with the dry BCG vaccine and the tularemia dry vaccine -- from 1:20 to 1:320 (an average of 1:111).

After 42 days following vaccination the allergic reaction was sharply positive in all the animals and to all the dilutions of tularin. The only exception were several pigs which did not react to 10 million tularin. Even here in the event of associated vaccination, in the test group of pigs high immunological indices were noted. In the group which was vaccinated with the liquid BCG vaccine and strain No 15 (attenuated) of the tularemia microbe they were higher.

As a result of the subcutaneous infection with 1000 Dclm of a virulent strain only 2 pigs died from tularemia. Of these, one was in the group which was inoculated with the dry tularemia vaccine (control) by using the 4-serrate scarificator, and one -- in the group inoculated by the same method with the liquid BCG vaccine and strain No 15 (reduced). Out of those which survived following infection, individual pigs had fever but not one of them lost weight.

For the purpose of exposing the periods of preservation of immunity against tularemia following the simultaneous vaccination against tularemia and tuberculosis, we set up a special test on 106 guinea pigs. The pigs were inoculated with a suspension of tularemia strain No 15 (attenuated) with a density of 1 billion and the liquid BCG vaccine (30 mg in 1 ml). Part of the animals were vaccinated with the 4-serrate scarificator, and the remaining -- by making incisions. Verification of the degree of immunity was carried out, just as in the previous tests, by means of the subcutaneous infection with 1000 Dclm of a virulent strain after 6 months following vaccination. As a result of the infection 6 out of the 35 pigs which were vaccinated separately died from tularemia, 8 out of the 36 which were inoculated with associated vaccines and 10 out of 35 which were vaccinated only against tularemia. The surviving pigs had fever for 10--20 days and some of them lost weight. Consequently, following the simultaneous vaccination the immunity against tularemia in the animals was maintained for 6 months to the same degree as in animals inoculated only with the tularemia vaccine. These data also support the feasibility of the combined application of the two vaccines used by us.

### Conclusions

1. The simultaneous cutaneous vaccination (separately or associated) against tularemia and tuberculosis creates a high immunity in guinea pigs against tularemia, and a specific resistance to subcutaneous infection with 1000 lethal doses of a virulent strain. Distinct immunological indices (allergic reaction and agglutination) in respect to tularemia were also noted in these pigs.
2. In the animals which were inoculated simultaneous with the two vaccines, the local and general reactions were practically no different from the reactions in pigs which were inoculated with monovaccines. This demonstrates the harmlessness of combined vaccination.
3. In guinea pigs which were vaccinated against tularemia and tuberculosis the immunity against tularemia was maintained over a period of 6 months (period of observation) to the same degree as in pigs which were inoculated with tularemia vaccine alone.
4. The data obtained demonstrates the feasibility of the simultaneous vaccination against these two infections with the complete compatibility of the vaccines and a mild reactogenicity, which makes it possible to recommend that the appropriate testing of vaccination against tularemia and tuberculosis be carried out on humans.

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Table 1

Results of the simultaneous (separate) vaccination of guinea pigs against tularemia and tuberculosis

Group	Vaccine	Average titer of agglutination reaction		Results of infection w/ 1000 Dc1m in 42 days after vaccination
		after 21 days	after 42 days	
First	Test	1:244	1:40	1/12
	Control	1:187	1:28	0/12
Second	Test	1:400	1:61	1/12
	Control	1:320	1:38	0/15

Note. Numerator - number of pigs which died from tularemia; denominator - total number of infected pigs.

Table 2

Results of vaccination against tularemia with strain No 15 (reduced) by using the 4-serrate scarificator

Dose of vaccine strain	Indices of agglutination reaction (avg. titers)		Results of infection with 1000 Dc1m
	after 21 days	after 42 days	
1 billion	1:92	1:60	0/6
100 million	1:74	1:40	0/8
10 million	1:54	1:26	4/10